Spatial constraints and seasonal conditions but not poaching pressure are linked with elevated faecal glucocorticoid metabolite concentrations in white rhino

Zoliswa N. Nhleko,[1,2,*] Andre Ganswindt,[3] Sam M. Ferreira,[2] Robert A. McCleery[4]

[1]Interdisciplinary Program in Ecology, University of Florida, Gainesville, FL 32611, USA.
[2]Savanna Node, Scientific Services, SANParks, Skukuza 1350, South Africa.
[3]Mammal Research Institute, University of Pretoria, Pretoria 0028, South Africa.
[4]Department of Wildlife Ecology and Conservation, University of Florida, Gainesville, FL 32611, USA.

[*]Correspondence to: Zoliswa N. Nhleko Interdisciplinary Program in Ecology, University of Florida, Gainesville, FL 32611, USA Email: nhlekozn@gmail.com

Abstract

Context. Due to considerable declines in African wildlife populations, most large African mammals are managed inside protected areas. Protected areas come in various sizes, and have different environmental features, climates and management strategies (i.e. 'hands-on' or 'hands-off') that can influence an animals' homeostasis. White rhinos (*Ceratotherium simum simum*) are found almost exclusively within protected areas where population sizes are driven by natural factors and poaching pressures.

Aims. Our aim was to understand the effect of natural and anthropogenic factors on the adrenocortical response of white rhinos within three protected areas. Specifically, we wanted to understand how poaching pressure, protected area size (<500 km^2), season (wet and dry) and rainfall patterns were responsible for driving adrenocortical activity in white rhino.

Methods. To understand the relationship between rhino adrenocortical responses and different environmental and anthropogenic stressors, we quantified glucocorticoid metabolites in faecal samples (fGCM) collected from four populations within three protected areas (i.e. two small parks, one big park) during the wet and dry seasons.

Key results. We found differences in seasonal fGCM concentrations, with a 42% increase during the dry season, and no differences in fGCM concentrations between the high and low poaching areas. Additionally, we found fGCM concentrations in samples from the small parks were respectively 38% and 42% higher than in samples from the large park during both the dry and wet seasons compared.

Conclusions. Our results suggest that white rhinos may experience physiological stress in smaller parks, especially during the dry season when resources are limited.

Implications. By mitigating stress associated with reduced access to resources and spatial constraints, managers may better promote the viability of large mammals in small protected areas.

Keywords: climate, fecal glucocorticoid metabolites, human activity, protected areas, poaching, season, stress, white rhino.

Introduction

The persistence of white rhino (*Ceratotherium simum*, rhino hereafter) is threatened by a growing suite of stressors [e.g. climate change (Ferreira *et al*. 2019), poaching (Thomas 2010), and space limitations (Balmford *et al*. 1995; du Toit 2006; Cousins *et al*. 2008)]. To maximise population growth of rhinos in South Africa, the South African National Parks (SANParks) manages them in both larger (>15 000 km^2) and smaller (<500 km^2) protected areas (Lindsey *et al*. 2017), with variable densities (0.01–1.0 rhino/ km^2), rainfall patterns (350–700 mm), and management strategies (e.g. supplemental feeding and hands-off) (Ferreira *et al*. 2017). Although it is clear that poaching and drought conditions are causing population declines (Gaillard *et al*. 2000; Nhleko *et al*. 2021), changes in the physiological status of rhinos in response to environmental stressors are less known but of great importance in ensuring individual health and thus population viability. Differences in rhino's physiological condition may be harder to detect than population declines, but understanding what causes them stress is equally important for developing strategies for conserving them.

Animals have several effective response mechanisms for dealing with perceived stressors, including the production and release of glucocorticoids (GCs) into the bloodstream (MacDougall-Shackleton *et al*. 2019; Palme 2019; Scheun *et al*. 2020). Specifically, GCs facilitate shifts in behaviour and physiology to limit the effects of stressors (Romero 2002; Ganswindt *et al*. 2010; MacDougall-Shackleton *et al*. 2019; Palme 2019; Scheun *et al*. 2020). Although the short-term release of GCs is adaptive in nature, a long-term elevation in GC concentrations can lead to deleterious consequences for individual fitness such as immune and reproductive suppression (Terio *et al*. 2004; Metrione *et al*. 2007; Viljoen *et al*. 2008a; Metrione and Harder 2011). A prolonged exposure to a stressful situation can even lead to a downregulation of adrenocorticoid output as a form of physiological protection measure, for example as shown in a rhino translocation study (Linklater *et al*. 2010). However, most other studies found that declines in food quality (Foley *et al*. 2001; Viljoen *et al*. 2008a) and hunting pressures elevated levels of GCs associated with stress in large animals (Bateson and Bradshaw 1997; Sforzi and Lovari 2000). Moreover, heavily hunted populations of other megaherbivores [e.g. elephants (*Loxodonta africana*)] have been shown to have higher GCs and lower reproductive output in poaching hotspots than in other areas (Gobush *et al*. 2008).

In the past, GC concentrations were determined from blood samples. However, the required handling and restraint of animals is usually perceived as a stressor and can lead to elevated GC concentrations (Ganswindt *et al*. 2010; Palme 2019). Quantifying respective GC metabolites from faecal samples provides an alternative non-invasive method for free-ranging animals (Ganswindt *et al*. 2010; Palme 2019; Scheun *et al*. 2020). Additionally, fGCM concentrations reflect the cumulative production of GCs over time, and are less affected by fluctuations in adrenal endocrine activity due to circadian rhythms (Touma and Palme 2005; Ganswindt *et al*. 2010). Although it is important to note that GCs themselves do not cause stress, they form part of an integral stress response, and so have been used as proxies for investigating physiological stress (Ganswindt *et al*. 2010; Ahlering *et al*. 2013; Mumby *et al*. 2015; Scheun *et al*. 2020).

Accordingly, the aim of this study was to investigate the relationship between rhino adrenocortical responses and different environmental and anthropogenic stressors. Specifically, using a non-invasive method for quantifying faecal glucocorticoid metabolites (fGCM), we wanted to (1) validate

our field methodology and understand the stability of fGCM concentrations for rhino post-defecation, and (2) correlate fGCM concentrations of four rhino populations to both environmental and anthropogenic challenges. We predicted that rhinos in areas with high poaching pressure would have higher fGCM concentrations compared with rhinos in areas with low poaching pressure due to their heightened perception of risk in these areas (Bateson and Bradshaw 1997; Sforzi and Lovari 2000; Vilela *et al*. 2020). Additionally, we predicted rhinos sampled during the dry season (August–September; when resources are limited) in the smaller protected areas that constrain this wide-ranging species (Clubb and Mason 2003, 2007; Metrione *et al*. 2007) would have higher fGCM concentrations.

Methods

Study sites

To understand the factors influencing fGCM levels in rhinos living under different environmental conditions, we collected faecal samples from populations within three protected areas of different sizes, management strategies and rainfall, poaching pressure and rhino population densities (Table 1). We chose a large park that allowed us to assess physiological conditions in areas with poaching and no poaching. We selected two smaller parks due to their similar size and the difference in rhino density, rainfall, and management strategies, which could influence rhinos' physiological responses.

Table 1. Parks used for the collection of white rhino dung samples for fGCM quantification.

Site	Size (km^2)	Population	Management	Rainfall (mm)	Samples wet	dry
Kruger	19 485	>2000	Hands off	500–700		
High poaching					54	26
Low poaching					53	29
Mokala	276	<100	Hands off	304–622	54	36
Marakele	290	<1000	Supplemental feeding	556–630	25	36

Parks are listed by size, estimated rhino population, management actions, rainfall, and the number of samples during wet and dry seasons.

We collected samples from a small population (20 to 100 animals) of rhinos (Ferreira *et al*. 2017) in Mokala National Park (Mokala, hereafter, 275 km^2), located in high-altitude grasslands [i.e. highveld of the Northern Cape Province of South Africa (Fig. 1)]. The park is semiarid and receives 350–558 mm of rainfall annually (Bezuidenhout *et al*. 2015). The winters are cool, with low temperatures averaging 1.1°C, and high summer temperatures averaging 32.8°C (Bezuidenhout *et al*. 2015). The underlying geology of the park consists of andesitic lavas of the Allanridge Andesite Formation in the north and the Karoo dolorite intrusions in the southern parts of the park (Bezuidenhout *et al*. 2015). The park has three dominant vegetation types, namely Kimberley Thornveld and Vaalbos Rocky Shrubland, which are part of the Savanna Biome, and the Northern Upper Karoo, which is part of the Nama Karoo Biome (Bezuidenhout *et al*. 2015). The dominant grass species in the park include *Eragrostis lehmanniana, Vachellia erioloba, Schmidtia pappophoroide, Eragrostis lehmanniana* and *Acacia mellifera* (Gertenbach 1983). Mokala was managed using a hands-off approach and there was no poaching occurring in the park at the time of the study.

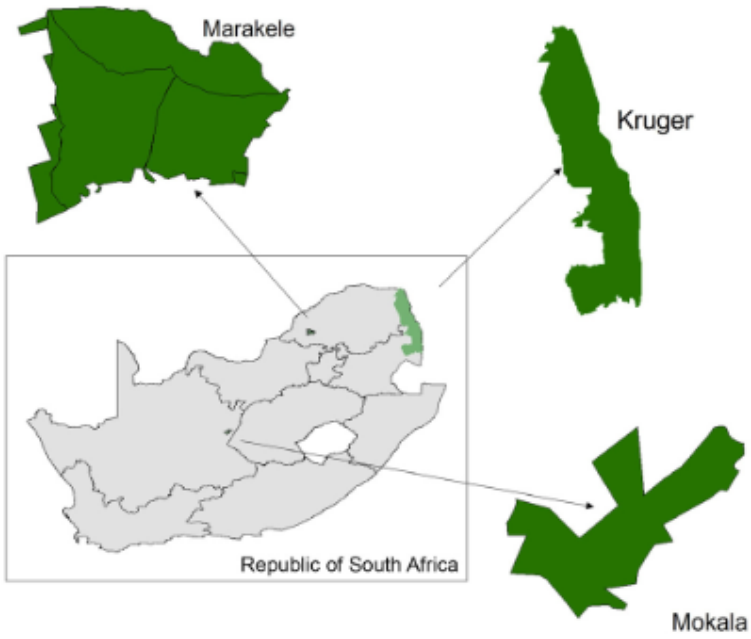

Fig. 1. Map of the three national parks in South Africa where white rhino faecal samples were collected in the wet and dry seasons of 2019.

We also collected faecal samples from rhinos in Marakele National Park (290 km^2, Marakele hereafter), located in the south-western part of the Limpopo province (Fig. 1). Marakele was home to a medium-sized population (100 to 500 animals) of rhino (Ferreira *et al*. 2017). The park receives 556–630 mm rainfall between October and March when high temperatures average 30°C (Novellie and Spies 2014). Winters are cool and dry, with frost occurring in low lying areas and low temperatures averaging 3°C (Novellie and Spies 2014). The underlying geology for the park is sandstone in the south-western and southern sections, with shale and mudstone in the west areas of the park (van Staden and Bredenkamp 2006). This gives rise to sandy soils on the sandstone and clay soils on the shale and mudstone (van Staden and Bredenkamp 2005). The park is situated in the Savanna Biome and the vegetation includes Sour Bushveld, Mixed Bushveld, Sourish Mixed Bushveld and North-Eastern Mountain Sourveld (van Staden and Bredenkamp 2006). The dominant grass species in the park include *Eragrostis curvula, Themeda triandra, Trachypogon spicatus, Eragrostis racemose* and *Setaria sphacelate* (van Staden and Bredenkamp 2005). Supplemental food was provided for rhinos in the dry season. There was no poaching occurring in the park during the time of the study.

Lastly, we collected faecal samples from a high and a low poaching area in Kruger National Park (19 485 km^2, hereafter Kruger), located in the Mpumalanga and Limpopo provinces (Fig. 1). Kruger is home to a large population (>2000 animals) of rhino (Ferreira *et al*. 2017; Nhleko *et al*. 2021). We used poaching records from 2018 (the year before our study) to find areas that had the highest and lowest number of poached rhinos. We used those poaching numbers to select our study sites; fewer than 50 rhinos were poached in the low poaching area compared with more than 150 rhinos in the high poaching area. The poaching levels for these two sections remained mostly unchanged during the year of our study (2019) and the following year (2020). Both areas were located in the southern parts of the park and border each other; however, we sampled from the interior portions of these

areas, which were separated by >50 km. The southern extent of Kruger lies within the lowveld bushveld climate zone, which receives 500–700 mm rainfall between October and March (Venter *et al*. 2003). The underlying geology of the park consists of granite and gneiss soils in the western parts, nutrient rich basalts in the eastern parts and Karoo sediments in the parts where the granite and basalts soils join (Venter *et al*. 2003). Vegetation on the southern region of park can be classified into two vegetation categories: (1) savanna woodlands on granite soils in the south where *Combretum* spp. trees are dominant; and (2) open grassy woodlands on the basalts in the south dominated by *Sclerocarya caffra* and S*enegalia nigrescens, Hyperthelia dissoluta, Eragrastis lappula, Erigrastis capensis* and *Themeda triandra* (Gertenbach 1983; Venter *et al*. 2003). Kruger is managed using a hands-off approach.

Sampling

We used fGCM concentrations to investigate the relationship between rhino physiological responses and different poaching rates, park sizes, seasons, and rainfall. We collected faecal samples from free-roaming rhinos (whose identities and sexes were unknown) in the wet (April 2019) and the dry (August and September 2019) seasons.

Stability of faecal glucocorticoid metabolite concentration post-defecation

Because several factors (i.e. temperature, bacterial enzyme) can influence fGCM concentrations found in samples, it is important to validate the technique used to monitor fGCM concentration for the species being studied (Touma and Palme 2005; Webster *et al*. 2018). This is done to ensure samples collected still contain concentrations of biologically relevant target agents (Webster *et al*. 2018). As such, we determined the stability of the fGCM concentrations post-defecation. To do this we collected a large faecal bolus from an individual rhino housed in the Skukuza bomas (enclosures) of Kruger National Park. We thoroughly mixed the material by hand (clad in rubber gloves) (Palme 2005) before freezing three subsamples as t = 0 control. We then divided the large faecal bolus into an additional 30 subsamples, which we placed outside in direct sunlight. We protected the samples using metal cages that excluded birds and mammals. Next, we removed and froze (−61.5°C) three subsamples at intervals of 30 min, 1hr, 2 h, 3 h, 6 h, 12 h, 24 h, 2 days, 4 days, and 7 days.

Faecal sample collection from free ranging rhinos

To collect faecal samples from the four different populations, we opportunistically searched for rhino middens with fresh samples in areas with high rhino activity such as footpaths, close to waterholes and on the sides of the road (Marneweck *et al*. 2018). We collected fresh (less than a week old – assessed by looking at discolouration and wetness) faecal samples from middens in the three protected areas (Kruger – in low and high poaching areas, Marakele and Mokala) at the end of the wet season, when biomass is highest (hereafter wet season; April 2019) and the dry season (August and September 2019). Faeces could not be allocated to individual rhinos because rhino identities were unknown. One downside to this opportunistic sampling is the potential for generating samples with a disproportionate number of pregnant females, which commonly have higher fGCM levels. However, because white rhinos reproduce throughout the year, we did not expect samples from pregnant females to bias our results. Where two or more fresh samples were found, we collected each sample from a different location on the midden where different individuals

defecate (i.e. territorial males defaecated in the centre of a midden, other rhinos defaecate around the periphery; Marneweck *et al*. 2018). The same collector retrieved samples from all sites and recorded the location of all middens where samples were collected. We collected all samples within a 2-week period at each site (2 weeks in Marakele, 2 weeks in Kruger and 2 weeks in Mokala) for each season (April 2019 for wet season, August and September for dry season sampling). We collected wet samples from the centre of the bolus to avoid the contaminated and dried out samples at the surface of the bolus. After collection, we placed the samples in individually labelled 30-mL bottles and stored them in a cooler box with ice packs until they could be frozen at the end of the day. To ensure we collected samples from different individuals at middens with multiple fresh samples, we collected faecal material from both the centre (where dominant males defaecate) and the periphery (used by females, juveniles, and subordinate males) (Marneweck *et al*. 2018). To reduce the potential for sampling from the same animal when collecting samples from unknown individuals, we collected samples 300–500 m apart, and used information on the spatial aspect of midden use found by Marneweck *et al*. (2018) to collect samples from different parts of the midden (Owen-Smith 1974; Marneweck *et al*. 2018). Samples were added to a cooler with ice packs immediately after collection, then frozen at −61.5°C at the end of each field day.

Steroid extraction and analysis

To extract GCMs from faecal samples we lyophilised, pulverised, and sieved the collected material through a wire-mesh strainer to remove fibrous material. We then mixed 0.050–0.055 g of faecal powder per sample with 3 mL of 80% ethanol, before placing the suspension in a vortex (Vortex Evaporator, Labconco, Kansas City, USA) for 15 min (Ganswindt *et al*. 2002). After the vortex, the samples were centrifuged at 352g for 10 min before the supernatants were decanted into microtubes and stored at −20°C until further analysis (Ganswindt *et al*. 2002).

We measured fGCM concentrations in each sample using an enzyme immunoassay (EIA) detecting fGCMs with a 5α-3β, 11β-diol structure. Detailed assay characteristics, including full descriptions of the assay components and cross-reactivities, have been provided by Touma *et al*. (2003), and the EIA has been shown to reliably measure alterations in fGCM concentrations in the species (Badenhorst *et al*. 2016). The sensitivity of the EIA was 2.4 ng/g faecal dry weight (DW). Serial dilutions of faecal extracts gave displacement curves that were parallel to the respective standard curves, with relative variation of the slope of the trend lines <2%. Intra-assay coefficients of variation (CV) of quality controls were 4.93% and 7.32%, and inter-assay CV were 10.22% and 14.74%. All analyses have been conducted at the Endocrine Research Laboratory, University of Pretoria, South Africa.

Statistical analysis

To determine a time-dependent alteration in fGCM concentration post-defaecation, fGCM concentrations for each subsample were expressed as percentages based on the triplicate mean value of subsamples exposed for 0 h (representing 100%). We graphically displaced variation in subsamples over time using the median and standard deviation (s.d.) to evaluate differences from t = 0.

Finding the samples from our four populations were not normal (Shapiro–Wilk test: $w = 0.80$, $P < 0.001$), we transformed measures of fGCM to ensure our data met the assumptions of normality.

Due to the importance of seasonality in understanding difference in fGCM, we first tested its influence on all our fGCM data using a generalised linear model with season as an explanatory variable. If we found no differences in seasons, we pooled the data by season; if there were differences, we accounted for seasonal effects in our remaining analyses by running separate analysis for each season. Next, to determine if the differences in fGCM could be explained by poaching intensity, we subset the data to the only area where poaching occurred (i.e. Kruger), and compared samples from low and high poaching areas, again using a generalised linear model. If we found no differences as a function of poaching, we pooled the Kruger data for future analyses. We evaluated seasonality and poaching by considering α levels <0.05 to be significant.

Finally, we compared the parsimony of three competing models (Anderson and Burnham 2004) to determine which provided the best explanation of variation in fGCM levels across all sites. For one model, we examined the size of the protected areas by comparing our two small study sites (Mokala and Marakele) with our larger site (Kruger). For our second model, we compared sites with more rainfall (Marakele and Kruger) to one with less (Mokala). For our final model, we consider possibility that the unique conditions (e.g. rhino density, management) at each our sites provided the best explanation of variation in fGCM levels (Table 1). We evaluated the parsimony of our three models and a null model using Akaike's Information Criterion corrected for small sample size (AICc). We considered the model with the lowest AICc to be the most parsimonious and regarded models within two AICc to be competing unless they were a configuration of the best model with an addition uninformative variable (Anderson and Burnham 2004). Additionally, we consider the variable in each competing model and consider them to be important predictors of fGCM levels if their 95% CI did not include 0 and their f-statistic was significant ($P < 0.05$). Finally, using spatial correlograms with non-parametric bootstrapping (BjØrnstad and Falck 2001), we found no evidence of spatial dependence in the residuals of the models of our best models for each season (Beale *et al*. 2010). We conducted our analysis using the packages ncf, MuMin, and ape (Paradis *et al*. 2004) on the R platform (R Core Development Team 2016).

Results

Stability of fGCM concentration post-defecation

Examining post-defecation over time we found no difference in fGCM concentrations over the course of 7 days (Appendix 1 in Supplementary Material). A maximum increase in fGCM concentration of 10.5% was recognised within the first hour, and an overall maximum increase in fGCM concentration of 21% after 7 days.

Comparison of fGCM from free ranging rhinos

We collected 90 samples from Mokala, 61 samples from Marakele, 82 samples from the low poaching area and 80 samples from the high poaching area of Kruger (Tables 1, 2). Examining samples from all our sites, we found a clear significant differences in the fGCM concentrations between the wet and dry season samples (β wet season = −0.57; 95% CI −0.78 to −0.36; $P < 0.001$), with an approximately 42% decrease in fGCM during the wet season (Fig. 2). Accordingly, we ran all our subsequent analyses separately for wet and dry seasons.

Table 2. The range of faecal glucocorticoid metabolite (fGCM) concentrations for white rhinos from different studies.

Site	fGCM levels[A] (µg/g DW)	References
Free ranging (Kruger)	0.03–1.14	This study
Free ranging (Mokala)	0.12–1.48	This study
Free ranging (Marakele)	0.03–1.46	This study
Hands-off orphan rearing	0.31–1.26	Fàbregas *et al.* (2020)
Hands-on orphan rearing	0.05–1.03	Fàbregas *et al.* (2020)
Before introduction	0.49–0.70	Fàbregas *et al.* (2019)
After introduction	0.40–0.94	Fàbregas *et al.* (2019)
Free ranging (Lapalala)	0.30–1.30	Badenhorst *et al.* (2016)

[A]All the fGCM samples in these studies were analysed in the same lab using the same method

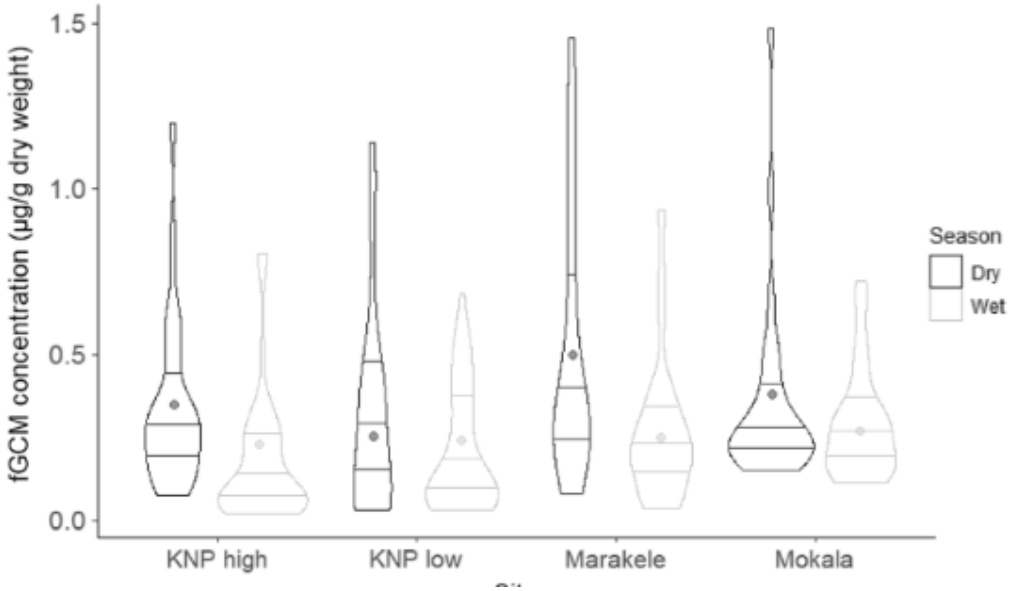

Fig. 2. Violin plots of faecal glucocorticoid metabolite (fGCM) concentrations of White Rhinos roaming at the three sampling sites in 2019. Values from the dry season are shown in grey and from the wet season in black. The lines represent the median and quartiles, and the dots represent the means.

To determine if variation *fGCM* could be explained by poaching levels, we used a subset of our data to compare areas with relatively high and low poaching in Kruger Park. We found no difference in the *fGCM* levels of rhinos between these areas of park during the wet (β = −0.03; 95% CI −0.11 to 0.04; P = 0.374) and dry seasons (β = 0.04; 95% CI −0.11 to −0.20; P = 0.573). Finding no differences in poaching intensity, we pooled together Kruger samples by season for further analyses.

Finally, we compared three different models to explain the variation in *fGCM* across our sites during both the wet and dry season. For both seasons the parsimonious model that explained the variation in *fGCM* levels was a model with a variable accounting for the size (large vs small) of the protected area (Table 3). The only competing model for each season was a model with one additional parameter that did not improve model parsimony, and was therefore not considered (Anderson and Burnham 2004). We found that fGCM levels were higher in smaller areas during both the wet (βsmall = 0.57; 95% CI 0.32–0.82; P < 0.001) and dry seasons (βsmall = 0.50; 95% CI 0.18–0.81, P = 0.002). Levels of fGCM were 37.5% higher on the small site during the dry season (small sites $\bar{x}$ = 0.44, large site $\bar{x}$ = 0.32) and 42% higher during the wet season (small sites $\bar{x}$ = 0.27, large site $\bar{x}$ = 0.19).

Table 3. Ranking of models used to explain seasonal (wet and dry season) variation in faecal glucocorticoid metabolite (fGCM) of white rhinos.

Season	Model	d.f.	AICc	ΔAICc
Wet	Size	3	479.3	0.00
	Site	4	479.9	0.62
	Rain	3	489.1	9.76
	Null	2	496.0	16.66
Dry	Size	3	297.0	0.00
	Site	4	297.8	0.89
	Rain	2	304.5	7.52
	Null	3	306.2	9.26

Size (small and large) and rainfall (high and low) at each site, as well as each area are considered separately (site) in models. A null model is also included. The degrees of freedom (d.f.) are reported, and models were ranked based on AICc (Akaike's Information Criterion corrected for small sample size) scores and relative difference in these scores (ΔAICc).

Discussion

In this study, we found differences in rhino's fGCM concentrations were best explained by differences in season and the size of their protected areas. Smaller protected areas make it easier to directly manage populations and limit threats from poaching (Clubb and Mason 2003; Cantú-Salazar and Gaston 2010); however, larger species that are adapted to roam over large spaces might be challenged by restricted movement (Clubb and Mason 2003, 2007; Metrione *et al.* 2007). Small parks may also be too small to encapsulate heterogeneous habitats and variable climatic conditions, limiting wildlife's access to refugia (Smit *et al.* 2020) or areas with higher quality food (Foley *et al.* 2001; Viljoen *et al.* 2008a; Islam *et al.* 2010) during unfavourable conditions (e.g. drought). Many larger mammals often use long-distance movement or migrations to access quality forage and water resources (Newmark 2008; Islam *et al.* 2010). Confinement to small protected islands, often surrounded by human dominated landscapes, may hinder the rhinos' ability to find favourable conditions, and ultimately trigger a stress response (Newmark 2008; Hetem *et al.* 2014).

The effect of small protected area size on the rhinos' fGCM concentrations appeared to be exacerbated by dry season conditions, with seasonally higher fGCM concentrations increasing an

additional 37.5% in rhinos in small protected areas compared with large ones. The dry season in southern Africa is associated with resource limitations for most wildlife (Shrader *et al.* 2006; Shrader and Perrin 2006), and likely exacerbates the restrictive nature of small protected areas. Other larger mammals (e.g. elephants) also appear to have elevated fGCM concentrations associated with the quality and quantity of food resources during the dry season (Foley *et al.* 2001; Viljoen *et al.* 2008a). However, only in large parks, like Kruger, do animals have the ability to mitigate insufficient food quality and quantity (e.g. elephant and buffalo [*Syncerus caffer*]) by shifting (up to ≥100 km) their activities to areas with greener vegetation and more rainfall (Abraham *et al.* 2019; Staver *et al.* 2019; Smit *et al.* 2020).

Contrary to our predictions and other research, we did not find differences in fGCM concentrations associated with poaching pressure levels. In fact, the overall fGCM levels for high and low poaching regions of Kruger were comparable to those for rhinos in other studies (Badenhorst *et al.* 2016; Fàbregas *et al.* 2019, 2020), further suggesting the lack of an elevated adrenocortical output in response to an imminent poaching pressure. Physiologically, the absence of higher fGCM levels could be a result of a negative feedback loop, where perceived stress over a prolonged period leads to a downregulation of adrenocortical activity (Linklater *et al.* 2010). However, studies have linked poaching and hunting activities to elevated fGCM concentrations in other species, like elephants (Gobush *et al.* 2008) and red deer (*Cevus elaphus*) (Bateson and Bradshaw 1997; Sforzi and Lovari 2000; Vilela *et al.* 2020).Rhinos' muted response to continued poaching may be due to habituation to continuous anthropogenic disturbance (Walker *et al.* 2006; Busch and Hayward 2009; Shutt et al. 2014). Alternatively, rhinos may rarely have non-lethal encounters with poachers. Rhinos travel in small groups (1–4) and are probably less likely than more gregarious herd animals (e.g. elephants and red deer) to have non-lethal encounters with humans and form memories about poaching events (Busch and Hayward 2009; Wiśniewska *et al.* 2022). The ability to associate risky events with places and stimuli may be critical for animals to form adaptive responses to poaching and other risks (Bradshaw *et al.* 2005; Busch and Hayward 2009; Wiśniewska *et al.* 2022).

Although there were clear differences in the population densities and management strategies at our two smaller protected areas, we found no evidence that these factors helped to explain the variation in the fGCM concentrations of white rhino. Specifically, differences in the density of white rhinos, supplemental feeding practices, average rainfall or environmental conditions (Table 1) had a marked influence on rhinos' physiological stress responses. Elevated levels of GCs have been associated with increases in population densities of small mammals, birds, and reptiles (Creel *et a*l. 2013). In our study, rhino population may not have reached the densities needed to trigger physiological stress responses. Alternatively, it is possible the supplemental feeding on the denser site (Marakele) offset the potential for food stress. However, the provision of food can increase the rates of contact between wildlife and humans as well as conspecifics, both of which can induce stress and increase rates of pathogen transmission (Murray *et al.* 2016). This increased contact between humans and rhinos may also habituate rhinos to humans and potentially increase their susceptibility to poaching.

Considerations

Although differences in fGCM concentrations may appear to be from environmental stressors, they may have also been a function of variation in the sample populations' age, body condition, reproductive stage, lactation, injury etc. (Ganswindt *et al.* 2003, 2010;

Rasmussen *et al.* 2008; Viljoen *et al.* 2008b; Breuner *et al.* 2013; Palme 2019). Results from the fGCM stability investigation suggested that the samples were fairly stable under the storage conditions of ambient temperatures, and that dung samples that had been in the field for up to 7 days would not influence our results. Previously, Ganswindt *et al*. (2012) found a 28.8% increase in fGCM levels in a stability experiment similar to ours. They concluded that changes of that magnitude did not affect their results (Ganswindt *et al*. 2012). Because we collected samples opportunistically, we were not able to collect information on age, sex, reproductive stage, or body condition of the rhinos from our sites. Studies have suggested that more robust results could be obtained by coupling fGCM concentrations with other measures (i.e. reproductive and body condition, glucose, fatty acids, and corticosteroid binding globulin) that influence concentrations of GCs in the blood stream (Breuner *et al*. 2013; Palme 2019). However, because some of these measures require invasive measures for sampling, these complementary measures are likely to increase animal stress, reduce sample size and greatly increase the costs of such a project.

Management implications

Due to the benefits of larger protected areas, when possible, managers should drop fences between neighbouring properties and enter into agreements for cooperative management to increase the amount of space available to wildlife. The creation of corridors to connect protected areas would also allow wildlife to move when conditions become unfavourable in a particular area (van Aarde and Jackson 2007). Still, one clear advantage of smaller protected areas for threatened and hunted species is their ability to provide a safe sanctuary from poaching. However, spatial constraints and resource availability for large mammals in smaller parks might impede some of the benefits gained, particularly during times of limited resources. Accordingly, the management of large animals in small, protected areas should be complemented with the provision of supplemental feed. However, the provision of food should avoid the aggregation of animals at feeding sites. This may be accomplished by spacing and moving feeding stations and providing feed at unpredictable times so animals cannot predict when and where the next feed will occur (Murray *et al*. 2016). To reduce contact between humans and wildlife, feed should be provided by a limited number of people, working as quickly and as silently as possible to minimalise disturbance. Managers should also ensure the population sizes of large mammals in smaller parks are kept low, allowing individuals to partake in their more natural ranging behaviours (Clubb and Mason 2003). By mitigating stress associated with reduced access to quality forage and water during the dry season, as well as spatial constraints faced by wildlife, managers can better promote the viability of large mammals in smaller protected areas.

Data availability. The data that support the findings of this study are available from the Science Manager at South African National Parks Mrs Judith Botha (judith.botha@sanparks.org), upon reasonable request.

Conflicts of interest. No conflicts of interest has been declared by the authors.

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